Aerobic exercise training reduces plasma endothelin-1 concentration in older women

Seiji Maeda, Takumi Tanabe, Takashi Miyauchi, Takeshi Otsuki, Jun Sugawara, Motoyuki Iemitsu, Shinya Kuno, Ryuichi Ajisaka, Iwao Yamaguchi, and Mitsuo Matsuda. Aerobic exercise training reduces plasma endothelin-1 concentration in older women. J Appl Physiol 95: 336–341, 2003. First published February 28, 2003; 10.1152/japplphysiol.01016.2002.—Endothelial function deteriorates with aging. On the other hand, exercise training improves the function of vascular endothelial cells. Endothelin-1 (ET-1), which is produced by vascular endothelial cells, has potent constrictor and proliferative activity in vascular smooth muscle cells and, therefore, has been implicated in regulation of vascular tone and progression of atherosclerosis. We previously reported significantly higher plasma ET-1 concentration in middle-aged than in young humans, and recently we showed that plasma ET-1 concentration was significantly decreased by aerobic exercise training in healthy young humans. We hypothesized that plasma ET-1 concentration increases with age, even in healthy adults, and that lifestyle modification (i.e., exercise) can reduce plasma ET-1 concentration in previously sedentary older adults. We measured plasma ET-1 concentration in healthy young women (21–28 yr old), healthy middle-aged women (31–47 yr old), and healthy older women (61–69 yr old). The plasma level of ET-1 significantly increased with aging (1.02 ± 0.08, 1.33 ± 0.11, and 2.90 ± 0.20 pg/ml in young, middle-aged, and older women, respectively). Plasma ET-1 concentration was markedly higher in healthy older women than in healthy young or middle-aged women (by ~3- and 2-fold, respectively). In healthy older women, we also measured plasma ET-1 concentration after 3 mo of aerobic exercise (cycling on a leg ergometer at 80% of ventilatory threshold for 30 min, 5 days/wk). Regular exercise significantly decreased plasma ET-1 concentration in the healthy older women (2.22 ± 0.16 pg/ml, P < 0.01) and also significantly reduced their blood pressure. The present study suggests that regular aerobic endurance exercise reduces plasma ET-1 concentration in older humans, and this reduction in plasma ET-1 concentration may have beneficial effects on the cardiovascular system (i.e., prevention of progression of hypertension and/or atherosclerosis by endogenous ET-1).

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IT IS GENERALLY ACCEPTED that loss of endothelial function not only is characteristic of diseases such as essential or secondary hypertension (35, 43), hypercholesterolemia (6, 54), and atherosclerosis (54) but also has been associated with advancing age (42). It has been reported that, independent of the presence of other pathological states, aging impairs endothelial function in the aorta and small resistance arteries (9, 12, 23, 33, 38). The alteration of endothelial function with aging may have important clinical implications in the pathogenesis of cardiovascular disease.

Vascular endothelial cells play an important role in the regulation of vascular activity by producing vasoactive substances, e.g., endothelin-1 (ET-1) and nitric oxide (27, 32, 50). ET-1 is a potent vasoconstrictor peptide produced by vascular endothelial cells (22, 27, 36, 50); in human vascular endothelial cells, it has a potent vasoconstrictor effect (27, 29). It has also been reported that systemic administration of an endothelin receptor antagonist significantly decreased systemic blood pressure and peripheral vascular resistance in healthy humans, strongly suggesting that endogenously generated ET-1 contributes to basal vascular tone in humans (10). Furthermore, ET-1 has potent proliferative activity in vascular smooth muscle cells; therefore, ET-1 has been implicated in the progression of atherosclerosis (16, 18, 27, 36). Our laboratory previously reported that plasma ET-1 concentration is increased in some human diseases (28, 31), e.g., chronic heart failure (11, 24, 39), acute myocardial infarction (31), and acute renal failure (47). Furthermore, pulmonary hypertension is associated with increased plasma ET-1 levels (5), and plasma ET-1 concentration correlates with disease severity (3, 13, 51). Therefore, the increase in plasma ET-1 level may have important clinical significance in the pathophysiology of some diseases. Furthermore, our laboratory also reported that plasma ET-1 concentration was significantly higher in middle-aged than in young humans (30), although plasma ET-1 concentration in older humans remains to be investigated. On the other hand, exercise training improves the function of vascular endothelial cells (8). Our laboratory recently showed...
that plasma ET-1 concentration was significantly decreased by aerobic exercise training in healthy young humans (21). It is of great interest and importance to study whether exercise training causes a decrease in plasma ET-1 concentration in older humans.

The purpose of the present study was to examine whether plasma ET-1 concentration increases with aging, especially in older humans, and is decreased by exercise training, even in older humans. We hypothesized that plasma ET-1 concentration increases with age, even in healthy adults, and that lifestyle modification (i.e., exercise) can reduce plasma ET-1 concentration in previously sedentary older adults. First, we measured plasma ET-1 concentration in healthy young women, healthy middle-aged women, and healthy older women. Second, in the healthy older women, we also measured plasma ET-1 concentration after 3 mo of aerobic exercise (cycling on a leg ergometer at 80% of age-predicted maximal heart rate, before and after the exercise training program. Their individual VT was calculated by using regression analysis of the slopes of CO₂ production, O₂ uptake, and minute ventilation plot (2, 7, 34). The older subjects submitted to a 3-mo exercise training program on a cycle ergometer for 30 min/day, 5 days/wk, at 80% of their individual VT.

**Methods**

**Subjects.** For experiment I, 39 healthy and untrained women (21–69 yr old) participated in a cross-sectional study. They were grouped into young (21–28 yr old, n = 16), middle-aged (31–47 yr old, n = 16), and older (61–69 yr old, n = 7) subjects. All subjects were normotensive (<140/90 mmHg). None of the women was taking medication on a regular basis at the time of the study. For experiment II, the older subjects performed an exercise intervention study. All seven older women were studied before and after 3 mo of aerobic exercise training. The study was approved by the Ethical Committees of the Institute of Health and Sport Sciences and the Institute of Clinical Medicine of the University of Tsukuba. The study conformed with the principles outlined in the Helsinki Declaration, and all subjects gave their written informed consent before inclusion in the study.

**Experimental design.** In experiment I, systolic blood pressure, diastolic blood pressure, and venous plasma ET-1 concentration at rest were measured in the young, middle-aged, and older groups. All participants were instructed to stop oral intake, including water, overnight 12 h before blood pressure measurement and ET-1 sampling in plasma. Blood pressure at rest was measured in duplicate, with subjects in the upright sitting position. All measurements were performed at a constant room temperature (25°C).

In experiment II, the older women completed an exercise intervention study. VT, resting systolic blood pressure, resting diastolic blood pressure, resting heart rate, and resting venous plasma ET-1 concentration were measured before and after 3 mo of aerobic exercise training in the older women. Before they were tested, subjects fasted for 12 h. Resting blood pressure and resting heart rate were measured in duplicate, with subjects in the upright sitting position. The measurements after the exercise training program were performed after ≥1 day of rest to rule out an acute effect from the most recent bout of exercise. All measurements were performed at a constant room temperature (25°C). Thus we controlled conditions preceding the measurements.

**Exercise test and exercise training in older subjects.** In experiment II, the older subjects performed symptom-limited ramp-fasion cycling exercise (after 2 min at 20 W, with 15-W increases every 1 min) until they felt exhausted or reached 85% of the age-predicted maximal heart rate, before and after the exercise training program. Their individual VT was calculated by using regression analysis of the slopes of CO₂ production, O₂ uptake, and minute ventilation plot (2, 7, 34). The older subjects submitted to a 3-mo exercise training program on a cycle ergometer for 30 min/day, 5 days/wk, at 80% of their individual VT.

**Measurement of plasma ET-1 concentration by sandwich-enzyme immunoassay.** Each blood sample was placed in a chilled tube containing aprotinin (300 kallikrein-inactivating units/ml) and EDTA (2 mg/ml) and then centrifuged at 2,000 g for 15 min at 4°C. The plasma was stored at −80°C until use. Plasma (1 ml) was acidified with 3 ml of 4% acetic acid, and immunoreactive ET-1 was extracted with a Sep-Pak C-18 cartridge (Waters, Milford, MA) as previously described (20, 21). The eluates were reconstituted with 0.25 ml of assay buffer and subjected to sandwich-enzyme immunoassay. The sandwich-enzyme immunoassay for ET-1 was carried out as previously described using immobilized mouse monoclonal antibody AwETN40, which recognizes the NH₂-terminal portion of ET-1, and peroxidase-labeled rabbit anti-ET-1 COOH-terminal peptide-(15–25) Fab′ (20, 21). The Fab′ fragment of this rabbit antibody was used as an enzyme-labeled detector antibody after being coupled with horseradish peroxidase. The intra- and interassay coefficients of variation of the ET-1 assay were 11 and 13%, respectively (25). Our laboratory previously reported that the lowest detection limit of this assay was 0.4 pg/ml for ET-1 (28). The plasma ET-1 levels in the present study were far beyond the lowest limit of detection with this assay (0.4 pg/ml) in all subjects.

**Statistics.** Values are means ± SE. To evaluate differences among young, middle-aged, and older women, statistical analysis was carried out by analysis of variance followed by Fisher's protected least significant difference test for multiple comparisons. To evaluate differences in the levels before and after exercise training in older women, Student’s t-test for paired values was used. P < 0.05 was accepted as significant.

**Results**

**Experiment I.** Table 1 shows the age and resting blood pressure of healthy young, middle-aged, and older women for the cross-sectional study. Systolic and diastolic blood pressures generally increased with aging (Table 1). Plasma ET-1 concentration significantly increased with aging (1.02 ± 0.08, 1.33 ± 0.11, and 2.90 ± 0.20 pg/ml in young, middle-aged, and older women, respectively; Fig. 1). Plasma ET-1 concentration was markedly higher in healthy older women than in healthy young or middle-aged women (Fig. 1). Indeed, plasma ET-1 concentration in older women was about

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**Table 1. Age and blood pressure in healthy young, middle-aged, and older women**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Young (n = 16)</th>
<th>Middle (n = 16)</th>
<th>Older (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>106 ± 3</td>
<td>121 ± 8§</td>
<td>127 ± 48</td>
</tr>
<tr>
<td>Diastolic</td>
<td>64 ± 2</td>
<td>71 ± 2§</td>
<td>79 ± 21§</td>
</tr>
</tbody>
</table>

*Values are means ± SE. Significantly different from Young: †P < 0.05 and §P < 0.01. Significantly different from middle: ‡P < 0.05 and &&P < 0.01.*
threefold higher than in young women and about two-fold higher than in middle-aged women. There was a significant positive correlation between systolic or diastolic blood pressure and plasma ET-1 concentration in the cross-sectional groups (systolic blood pressure vs. ET-1: \( r = 0.490, P < 0.05 \); diastolic blood pressure vs. ET-1: \( r = 0.488, P < 0.05 \)).

**Experiment II.** All seven older women completed the exercise intervention study. Table 2 shows the physiological parameters in the older women before and after 3 mo of aerobic exercise training. There were no significant differences in body weight and body mass index before and after exercise training (Table 2). Systolic and diastolic blood pressures at rest significantly decreased after exercise training, whereas heart rate at rest was not different (Table 2). After exercise training, individual VT during the exercise test significantly increased (Fig. 2). These results suggest that 3 mo of exercise training in the older women caused physiological effects, i.e., effects of exercise training, as evidenced by the decrease in blood pressure at rest and the increase in individual VT during the cycle exercise test. Figure 3 shows the resting plasma ET-1 concentration in the older women before and after exercise training. The plasma concentration of ET-1 significantly decreased after exercise training (2.90 ± 0.20 vs. 2.22 ± 0.16 pg/ml, \( P < 0.01 \); Fig. 3). There was a tendency for a positive correlation between the changes in systolic or diastolic blood pressure and the changes in ET-1 after exercise training (systolic blood pressure vs. ET-1: \( r = 0.479 \); diastolic blood pressure vs. ET-1: \( r = 0.590 \)), but the correlation was not statistically significant.

**DISCUSSION**

In the present study, we measured plasma ET-1 concentration in healthy young women, healthy middle-aged women, and healthy older women. Furthermore, the older women participated in an exercise intervention study, and we also measured the plasma ET-1 concentration after 3 mo of exercise. The plasma ET-1 concentration significantly increased with aging; i.e., plasma ET-1 concentration was markedly higher in healthy older women than in healthy young or middle-aged women (by ~3- and 2-fold, respectively). We also demonstrated that regular exercise in the older subjects significantly decreased plasma ET-1 concentration. Because it is considered that circulating plasma ET-1 may mainly originate from vascular endothelial cells (27), it is possible that the increased production of ET-1 in vascular endothelial cells of older humans is decreased by regular aerobic exercise; there-

### Table 2. Effects of exercise training in older women

<table>
<thead>
<tr>
<th></th>
<th>Before Training</th>
<th>After Training</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height, cm</td>
<td>152 ± 2</td>
<td>152 ± 2</td>
<td>0.85</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>51 ± 2</td>
<td>51 ± 2</td>
<td>0.87</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22 ± 1</td>
<td>22 ± 1</td>
<td>0.20</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>76 ± 4</td>
<td>72 ± 3</td>
<td>0.03</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>127 ± 4</td>
<td>112 ± 3</td>
<td>0.01</td>
</tr>
<tr>
<td>Diastolic</td>
<td>79 ± 2</td>
<td>65 ± 2</td>
<td></td>
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</tbody>
</table>

Values are means ± SE.
fore, this phenomenon as a result of regular aerobic exercise may produce beneficial effects on the cardiovascular system (i.e., prevention of progression of hypertension and/or atherosclerosis by endogenous ET-1).

Our laboratory previously reported that plasma ET-1 concentration was significantly higher in middle-aged than in young humans (30). The present study demonstrated that plasma ET-1 concentration was markedly increased in older women; i.e., plasma ET-1 concentration significantly increased with age, even in healthy humans. Endothelial function deteriorates with aging (9, 12, 23, 33, 38, 42). Thus, because ET-1 is produced by vascular endothelial cells (22, 27, 36, 50), it is considered that the increase in ET-1 in older women may be a factor in aging-induced loss of endothelial function.

The conclusions drawn in the present study come from plasma ET-1 levels. It has been demonstrated that a twofold increase in plasma ET-1 concentration by the intravenous infusion of exogenous ET-1 significantly increased renal and systemic vascular resistances, suggesting that circulating plasma ET-1 may have biological actions on the cardiovascular system (19). However, it is generally accepted that ET-1 acts predominantly in an autocrine and paracrine manner, and its secretion by endothelial cells is polarized toward the underlying vascular smooth muscle (27, 48). Consequently, plasma levels are largely the results of spillover from vascular endothelium into the bloodstream. Indeed, only ~20% of generated ET-1 is secreted luminally (52). Therefore, because plasma ET-1 concentration is very low and ET-1 is not a circulating hormone, it is considered that tissue ET-1 is more important than circulating ET-1. Thus the present study has the following limitations: 1) it is unclear whether vascular ET-1 content increases with age in humans, and 2) it is unclear whether exercise training reduces vascular ET-1 content in older humans.

Taddei et al. (41) showed that plasma ET-1 levels do not differ between age-matched normotensive and hypertensive subjects, whereas when the biological action of ET-1 was blocked, Taddei et al. noted a greater influence of ET-1 on vascular tone in hypertensive subjects, suggesting a possible role for ET-1 in the pathogenesis of hypertension and/or its complications. On the other hand, pulmonary hypertension is associated with increased plasma ET-1 concentrations (5), and the plasma level of ET-1 correlates with disease severity (3, 13, 51). More recently, it has also been reported that endothelin receptor blockade causes a decrease in mean pulmonary arterial pressure in patients with pulmonary hypertension (1). Therefore, because it is considered that circulating plasma ET-1 mainly originates from vascular endothelial cells (27) and the magnitude of spillover of ET-1 into the plasma reflects the magnitude of ET-1 production originated from the vascular endothelial cells, it is possible that plasma ET-1 levels reflect the tissue ET-1 levels in some pathophysiological conditions. Therefore, in the present study, it is likely that alteration of the circulating ET-1 by exercise training may reflect alteration of ET-1 production by vascular endothelial cells or tissue ET-1 content in the vessels in the older humans.

It has been reported that patients with essential hypertension have increased vascular ET-1 activity, which may be of pathophysiological relevance to their increased vascular tone (4). An increased vascular response to ET-1 has also been reported in an animal model of hypertension (26, 40). Furthermore, ET-1 has potent proliferative activity in vascular smooth muscle cells and has, therefore, been implicated in the progression of atherosclerosis (16, 18, 27, 36). It has also been reported that ET-1 expression increases in human atherosclerotic lesions (18, 49, 53). On the other hand, it is well known that regular exercise produces beneficial effects on the cardiovascular system. Chronic exercise reduces blood pressure in patients with moderate hypertension (37, 45, 46). The aging-induced reduction of arterial compliance causes an increase in systolic blood pressure, whereas exercise training prevents this reduction in arterial compliance (14, 15, 44). It has also been reported that exercise training has a favorable effect on the development of atherosclerosis (17). However, the precise mechanisms by which exercise training reduces blood pressure and the risk of atherosclerosis have not been fully determined. Our present study demonstrated that the aging-related great elevation in endogenous ET-1 significantly decreased after exercise training in older humans. We also observed a reduction of blood pressure after exercise training in the older women with a reduction of plasma ET-1. ET-1 has not only potent constrictor activity, but also proliferative activity, in vascular smooth muscle cells (16, 18, 22, 27, 36, 50). Therefore, it is considered that the decrease in ET-1 production in endothelial cells by exercise training would be partly involved in the exercise training-induced beneficial effects on the cardiovascular system in older humans.

It is well known that exercise training induces an increase in plasma volume in some conditions. In the present study, there was no significant difference in the hematocrit in the older women before and after exercise training (45.7 ± 0.5 vs. 45.2 ± 0.7%). Therefore, it is considered that the reduction in plasma ET-1 levels with the present exercise intervention simply does not represent a dilution relative to an exercise training-induced increase in plasma volume.

In animal studies, endothelin receptor antagonists improve various cardiovascular diseases, such as chronic heart failure, hypertension, and pulmonary hypertension (27). It is of great interest and importance to study whether exercise training causes a decrease in plasma ET-1 concentration in patients with cardiovascular disease, such as hypertension and atherosclerosis. Such studies will provide important information on whether endogenous ET-1 is involved in the exercise training-induced beneficial effects in patients with these cardiovascular diseases.

In conclusion, we demonstrated that plasma ET-1 concentration significantly increased with aging, being markedly higher in older women than in young or middle-aged women (by ~3- and 2-fold, respectively).
The present study also demonstrated that regular aerobic-endurance exercise in older humans significantly decreased plasma ET-1 concentration. Because ET-1 has potent constrictor and proliferative activity in vascular smooth muscle cells and has been implicated in the regulation of vascular tone and the progression of atherosclerosis, we propose that the decrease in production of ET-1 by exercise training may be partly involved in the beneficial effects of chronic exercise on the cardiovascular system in older humans.

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